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Influence of nanogel additive hydrophilicity on dental adhesive mechanical performance and dentin bonding

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Abstract

Objective—To assess the influence of hydrophilicity of reactive nanogels on the mechanical performance of dental adhesives and microtensile bond strength (μ TBS) to dentin after 24 h or 3 months of aging.

Methods—A series of three nanogels were synthesized: NG1—IBMA/UDMA; NG2—HEMA/BisGMA; NG3—HEMA/TE-EGDMA. The nanogels were dispersed in solvent, HEMA or BisGMA/HEMA. The degree of conversion (DC) of the materials was measured and the flexural modulus of these polymers was evaluated in dry or wet conditions. For μ TBS analysis, a model adhesive was used without nanogel (control) or with the incorporation of nanogels. μ TBS was evaluated after storage in distilled water for 24h or 3 months. The analysis of the fracture was performed after μ TBS testing. Data were analyzed using ANOVA and Tukey's test ($\alpha = 0.05$).

Results—Water significantly increased the modulus of NG1 and NG2 dispersed in solvent, while significantly decreased the stiffness of NG3. All polymers dispersed in HEMA and Bis-GMA/HEMA had significantly lower modulus when stored in water. NG2 showed the highest DC in solvent and BisGMA/HEMA. In HEMA, NG1 and NG3 produced the highest DC. After three months, NG2 showed the best μ TBS. The μ TBS of NG2-containing adhesive resin significantly increased after 3 months, while storage had no effect in the control group, NG1 and NG3.

Significance—The more hydrophobic IBMA/UDMA nanogel showed higher bulk material mechanical property results, but the best dentin bond strength values, and notably strength values that improved upon storage, were obtained with the amphiphilic nanogel based on BisGMA/HEMA.

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Keywords

Nanogel; Dental material; Hydrophilicity; Adhesion; Hydrolysis

1. Introduction

Although high quality adhesive systems are available for applications in restorative dentistry, the great immediate results of bond strength typically decline with storage time. The adhesive interface remains the weakest component of dental restorations based on varied degrees of degradation over relatively short times [1]. This seems to be due to several factors such as substrate characteristics, technical limitations, aging conditions in the oral environment (i.e., temperature, moisture and chewing load) and the composition of adhesives [2–6].

To ensure appropriate hybridization of wet collagen matrix, increasing concentrations of hydrophilic and ionic monomers have been added to adhesives [7]. Furthermore, simplified adhesive systems also have a large concentration of solvents and hydrophilic monomers [8,9], which interferes in the polymerization and decreases the degree of conversion [10–12]. The addition of hydrophilic monomers forms a network with low cross-linking density, and increases water sorption/solubility and resin plasticization [13,14]. These factors are thought to contribute directly to the hydrolytic and potential enzymatic degradation of the polymer resin.

Different strategies have been developed to control and prevent the degradation of the hybrid layer, such as ethanol wet bonding [13–17], chlorhexidine [18], and antioxidants/crosslinking agents [19–23]. Moreover, good results have also been obtained using nanomaterials in adhesive formulations [24].

Nanotechnology in the form of reactive nano-scale prepolymeric particles that can be swollen by monomer has attracted substantial interest due to the versatile structures with multiple applications in the drug delivery, tissue engineering and polymer composites [25,26]. While a large number of biomedical applications involve nanogels as freely dispersed particles, recent studies have applied nanogels as functional fillers or additives in the preparation of nano-composite polymer networks [24–27].

In general, incorporation of nanogels in dental adhesive systems and composites reduced shrinkage and improved mechanical properties such as flexural modulus and flexural strength, both in dry and wet conditions, due to the strengthening of the polymeric network by the presence of the crosslinked particles [24]. Furthermore, water solubility was reduced, and short-term bond strength to dentin was improved significantly with the inclusion of the nanogels, without need to modify the existing application techniques [24].

Despite the use of nanogels as an interesting option for the modification of dental materials, the effects of comonomer combinations within the nanogels that produce different levels of hydrophilicity have not been studied with regard to adhesive formulations and bonding to a dentin substrate. The interaction between materials and substrates is very important for the

establishment of efficient and effective adhesive bonds. It is reasonable to expect that specific types of nanoparticles may be able to influence the properties and durability of polymers. Thus, the aim of this study was to compare three nanogels that systematically differ in terms of hydrophilicity and assess how the incorporation of these nanostructures into dental adhesives will influence the mechanical performance of materials and bond strength to dentin over early storage times. The null hypotheses to be tested were: (1) nanogels with different hydrophilicity would not affect the mechanical performance when exposed to water; (2) nanogels with different hydrophilicity would not interfere with dentin bond strength.

2. Materials and methods

2.1. Nanogel synthesis

Three nanogel (NG) copolymers were synthesized at a 70:30 molar ratio of: isobornyl methacrylate (IBMA; TCI America, Portland, OR, USA) and urethane dimethacrylate (UDMA; Sigma–Aldrich Co., St. Louis, MO, USA) (NG1); 2-hydroxyethyl methacrylate (HEMA; TCI America) and bisphenol A glycerolate dimethacrylate (BisGMA; Essstech, Essington, PA, USA) (NG2); 2-hydroxyethyl methacrylate (HEMA) and tetraethylene glycol dimethacrylate (TE-EGDMA; TCI America) (NG3). 2-Mercaptoethanol (ME; Sigma–Aldrich) was added (10 mol% for NG1, 40 mol% for NG2, and 15 mol% for NG3 relative to monomers) as a chain-transfer agent to avoid macrogelation, control molecular weight/nanogel particle size, and provide sites for post-polymerization refunctionalization with reactive groups. Free radical polymerization was conducted in solution (six-fold excess for NG1, eight-fold excess for NG2, seven-fold excess for NG3 of methyl ethyl ketone (MEK; Fisher Scientific, Waltham, MA, USA) relative to monomer) with 1 wt% 2,2'-azobisisobutyronitrile (AIBN; Sigma–Aldrich) as thermal initiator. A 100 mL round-bottom flask was used as the reactor with monomer batch sizes of approximately 10 g with reaction conditions of 80 °C and a stirring rate of 200 rpm. Methacrylate conversion during nanogel synthesis was calculated from mid-IR spectra (Nicolet 6700, Thermo Scientific, Waltham, MA, USA) before and after polymerization.

Nanogels were purified by precipitation from hexanes (10-fold excess; Fisher Scientific) and filtration. Resulting precipitates were re-suspended in dichloromethane (BDH Chemicals, VWR Analytical, Radnor, PA, USA) for NG1 and NG3, and acetone (Fisher Scientific) for NG2, and reacted at room temperature with a 10 mol% for NG1, 10 mol% for NG2, and 15 mol% for NG3 of 2-isocynoethyl methacrylate (IEM; TCI America) with a trace amount of dibutyltin dilaurate (Sigma–Aldrich) as catalyst. The polymer precipitation method was repeated to isolate the methacrylate-functionalized reactive nanogel. Residual solvent was removed completely under vacuum until the nanogels were obtained as dry powders.

2.2. Nanogel particle characterization

Polymeric nanogels were characterized by triple-detector (refractive index, viscosity, light scattering) gel permeation chromatography—GPC (GPCmax; Viscotek, Malvern Instruments, Malvern, UK) in tetrahydrofuran (EMD Millipore, Billerica, MA, USA). The glass transition temperature (T_g) of nanogel powders was determined by dynamic

mechanical analysis (DMA 8000, PerkinElmer, Waltham, MA, USA) by sandwiching 10 mg of nanogel in a thin metallic pocket that was then subjected to single cantilever cyclic displacement of 50 μm at 1 Hz. The nanogel was heated to 150 °C with $\tan \delta$ data collected as the sample was cooled to 0 °C at 2 °C/min in air.

2.3. Mechanical strength characterization

The flexural modulus was evaluated for all nanogels dispersed only with inert solvent or in HEMA or BisGMA/HEMA. For the solvated nanogel groups, NG1 was dispersed in *N,N*-dimethylformamide (DMF) (Sigma–Aldrich), while NG2 and NG3 were dispersed in ethanol (Fisher Scientific), according to their solubility characteristics. Slight differences in the weight percentages of nanogels were established according to the specific viscosity characteristics of the blends. This was done as needed to normalize the viscosities between formulations and avoid difficulties related to flow in handling and specimen preparation. When in solvent, nanogels were dispersed at 50:50 wt% NG:solvent; when nanogels were dispersed in HEMA, a 50:50 wt% NG:monomer proportion was used for NG2 and NG3, while a 48:52 wt% NG:monomer formulation was used for NG1; and when the nanogels were dispersed in BisGMA/HEMA (40:60 wt%) in a 40:60 wt% NG:resin ratio. Viscosity measurements of the nanogel-modified materials were performed using a cone-plate digital viscometer (CAP2000+; Brookfield, Middleboro, MA, USA) at ambient temperature.

Flexural modulus tests were performed using bar specimens ($n = 8$) with dimensions of 25 mm $\times$ 2 mm $\times$ 2 mm, light-cured between glass slides in an elastomer mold by exposure to UV light at 60 mW/cm² for 2 min on each side (mercury arc lamp 365 nm—Acticure 4000, EXFO, Richardson, TX, USA). 2,2-Dimethoxy-2-phenylacetophenone (Sigma–Aldrich) was used at 0.2 wt% as an efficient UV photoinitiator to obtain the bulk adhesive resin polymers with high conversion to allow meaningful analysis of the effect of the different nanogels in these materials. The degree of conversion (Nicolet 6700) of all the bar specimens was evaluated following the preparation of the samples, before storage. Methacrylate conversion was calculated by following the area of the methacrylate absorption band in near-IR (6165 cm⁻¹) before and after photoactivation. The bars were stored in an oven (Single-Wall Transite Oven; Blue M Electric Company, Blue Island, IL, USA) overnight at 37 °C and then randomly divided into two groups ($n = 8$): dry storage condition and wet storage condition. After one-week additional storage in dark containers (dry or in distilled water) at room temperature, which provided coordinated testing of dry and wet polymers after sufficient time for near-equilibrium water uptake in the wet storage samples, the flexural modulus was obtained in three-point bending on the MTS testing machine using a span of 20 mm and a cross-head speed of 1 mm/min (MTS Mini Bionix II, MTS, Eden Prairie, MN, USA).

2.4. Dentin-bonding study

A dentin-bonding study was conducted with a model ethanol-solvated (30 wt%) BisGMA/HEMA (40:60 wt%) etch-and-rinse 1 bottle adhesive as a control or containing nanogels 1, 2 or 3 at 40 wt% relative to the BisGMA/HEMA monomer content. The 40 wt% nanogel loading level was selected to provide near confluent nanogel packing in the adhesive resin upon evaporation of a majority of the solvent [26]. Camphorquinone (Sigma–Aldrich) and

ethyl 4-*N,N*-dimethylaminobenzoate (Sigma–Aldrich) were added at 0.2 and 0.6 wt%, respectively, as co-initiators.

Forty extracted human molars were obtained under approval of the institutional ethics committee (protocol 022/2014) and stored in distilled water at 4 °C no longer than 6 months. The roots were removed 2 mm beneath the cement-enamel junction (CEJ), while the occlusal enamel was cut 2 mm above the CEJ using a slow-speed water-cooled diamond saw (Isomet, Buehler; Lake Bluff, IL, USA), exposing a flat surface of dentin. The exposed dentin surface was abraded before bonding with a 600-grit SiC paper for 60 s under running water, with the intent to create a standardized smear layer.

Prepared teeth were randomly divided into four groups ($n = 10$) according to adhesive systems (with or without NG). The samples were subjected to acid etching with 35% phosphoric acid (Scotchbond Universal Etchant, 3M ESPE) for 15 s. All adhesive systems were applied in 2 coats. Light activation was performed for 10 s using a LED Elipar Deepcure-S lamp (1000–1200 mW/cm², 3M ESPE, St. Paul, MN, USA). Composite buildups were constructed using Filtek Z-100 (3M ESPE) in 2 layers (each layer 2 mm thick). Approximately 20–25 sticks (bonding area 1×1 mm²) were obtained from each molar by sectioning the bonded teeth. The sticks from the periphery showing remaining enamel were excluded. Half of the sticks were tested after 24 h of restoration and the other half after 3 months of storage in distilled water at 37 °C.

The sticks were fixed to a jig using a cyanoacrylate gel and tested to failure on MTS testing machine at a crosshead speed of 1.0 mm/min. The cross-sectional area of the sticks was measured before failure with a digital caliper. The μ TBS of the sticks from the same bonded tooth were averaged and used for the statistical analysis as the statistical unit. Means and standard deviations were calculated and expressed in MPa. A small number of the bonded specimens failed prematurely and were not included in the statistical analysis. This defect exclusion is supported by the very limited numbers involved and that the range of bond strengths achieved was all relatively narrow and well above that associated with spontaneous failure.

The fractured specimens were analyzed using SEM JSM-5600LV (JEOL; Tokyo, Japan). In brief, the specimens were paired and mounted on aluminum stubs and subsequently dehydrated in silica gel. Finally, the specimens were gold-sputter coated and examined using SEM, operated at 15 kV. The fractures were classified as adhesive, cohesive in composite, cohesive in adhesive, cohesive in dentin or mixed.

2.5. Sorption and solubility and contact angle

Disc specimens (1×5 mm, $n = 5$) were produced between glass slides with a model ethanol-solvated BisGMA/HEMA (40:60 wt%) etch-and-rinse 1 bottle adhesive as a control or containing nanogels 1, 2 or 3 at 40 wt% relative to the BisGMA/HEMA monomer content. Camphorquinone (Sigma–Aldrich) and ethyl 4-*N,N*-dimethylaminobenzoate (Sigma–Aldrich) were added at 0.2 and 0.6 wt%, respectively, as co-initiators. Ethanol was included in the adhesive resin at 12 wt% to simulate a adhesive containing residual solvent [24]. The

samples were photoactivated for 2 min on each side by exposure to visible light at 100 mW/cm² (mercury arc lamp 320–500 nm—Acticure 4000, EXFO).

Water sorption (WS) and solubility (SL) were calculated as: $WS = m_2 - m_3/V$ and $SL = m_1 - m_3/V$, where m_1 is the initial adhesive polymer mass before water immersion, m_2 is the wet mass of the surfaceblotted water-equilibrated polymer, and m_3 is the dry mass of the polymer after 7 days' storage in a desiccator. The water contact angle of the 12% ethanol-containing pre-immersion discs was measured by means of a goniometer (Ramé-Hart Instruments Co., Netcong, NJ, USA). One drop of distilled water was placed on the surface of the material, then a profile image was recorded and the contact angle ($n = 3$) measured (DROPimage Advanced; Ramé-Hart).

2.6. Statistical analysis

The data were statistically analyzed by equal variance and Kolmogorov–Smirnov normality tests and, after proving the normality of data and homogeneity of variances, the results were analyzed using one-way ANOVA (nanogel, for degree of conversion) and two-way ANOVA (nanogel and condition – dry or wet, for flexural modulus; and nanogel and storage time – 24 h and 3 months, for microtensile test) and Tukey's test ($\alpha = 0.05$). For flexural results and degree of conversion, the analyses were performed for each dispersion medium separately (solvent, HEMA and BisGMA/HEMA).

3. Results

Methacrylate conversion of approximately 90% was achieved for NG1 after about 4 h of reaction, 78% for NG2 after 2.5 h reaction, and 90% for NG3 following approximately 3 h of reaction. Number and weight-averaged molecular weight (M_n and M_w , respectively), polydispersity index (PDI), hydrodynamic radius (R_h), and Mark–Houwink α exponent (MH- α) were obtained by GPC analysis (Table 1). Dynamic mechanical analysis of NG1, NG2 and NG3 provided bulk nanogel Tg values of 96 °C, 55 °C and 40 °C, respectively (Table 1).

As expected, viscosities of the monomer-based nanogel nanogels were substantially higher than that of the solvent-based dispersions (Table 2). The use of 40 wt% nanogel loading in the BisGMA/HEMA resin provided viscosities that were only modestly higher than those obtained with the higher nanogel loading levels used with HEMA as the dispersant. Based on its composition and greater hydrodynamic radius, NG1 consistently provided formulations with the highest viscosities.

The degree of conversion (DC) ranged from 82.9% to 91.4% (Table 2). For solvent and BisGMA/HEMA groups, NG2 had the highest DC. When mixed in HEMA, NG1 and NG3 were not statistically different and showed the highest DC.

Results for the polymeric flexural modulus (Table 2) demonstrate that the interaction between the factors was significant for all dispersion media (solvent, HEMA, Bis-GMA/HEMA; $p < 0.001$). For the dry storage condition, NG2 presented the highest modulus in

solvent and dispersed in HEMA. BisGMA/HEMA control showed the highest modulus compared to nanogels groups dispersed in BisGMA/HEMA 40:60 wt%.

Under wet storage conditions, NG1 presented the highest modulus in HEMA and BisGMA/HEMA. When solvent dispersed, NG1 and NG2 were not statistically different, and showed the highest modulus values.

After storage in water, NG1 and NG2 solvent groups showed significantly higher modulus in wet conditions compared to dry condition, while NG3 had a significant drop when saturated with water. When mixed with HEMA or Bis-GMA/HEMA, all nanogels groups including the nanogel-free controls showed significantly lower modulus in wet conditions compared to dry condition. The wet moduli for all the nanogel-modified samples were significantly greater than the HEMA control while only BisGMA/HEMA with NG1 provided a higher wet modulus than the resin control.

The water contact angle associated with the NG1 ethanol-solvated BisGMA/HEMA adhesive was significantly greater than those with NG2, NG3 and the control group (Table 3) with the transition between the more hydrophobic NG1 to the amphiphilic NG2 to the more hydrophilic NG3 showing the expected progressive reduction in contact angle. NG1 and NG2 yielded reduced water sorption with NG1 also providing decreased solubility. The inclusion of even the most hydrophilic nanogel (NG3) to the dental adhesive did not increase the sorption or solubility properties relative to the control.

In the 24 h μ TBS testing (MPa, Table 3), the control group showed the highest bond strength value, but not statistically different from group with NG2. After 3 months of storage in distilled water, NG2 presented the highest results. NG2 had statistically higher bond strength after 3 months, compared to 24 h. Control, NG1 and NG3 maintained their values after storage.

The failure (Fig. 1) was predominantly adhesive for control groups and NG3 at 24 h and after 3 months. For NG1 and NG2, the failure pattern changed from mixed to adhesive after storage.

4. Discussion

According to the GPC analysis (Table 1), the three nanogels have particles with size of approximately 10 nm and globular structure such that they potentially can be dispersed into dentinal tubules and interfibrillar collagen spaces [24,25,28]. The roughly spherical or globular structure of the nanogel is confirmed by the Mark–Houwink α exponent (0.28–0.36) as compared with a typical value of approximately 0.7 associated with a random coil linear polymer structure [29]. The higher concentration of chain transfer agent and solvent used in the synthesis of NG2 was necessary to avoid macrogelation. The viscosity results (Table 2) demonstrate that choice of the nanogel and its loading level, the resin into which it is added and any solvent used as temporary diluent can provide a wide range of adhesive viscosity. The thermal scan of the bulk nanogel powder samples by dynamic mechanical analysis provided Tg values of 96 °C, 55 °C and 40 °C, respectively for NG1, NG2 and NG3. Isobornyl methacrylate contributes toward the relatively high Tg of NG1 while the

BisGMA crosslinking component of NG2 increases its T_g compared with TE-EGDMA in NG3. The T_g of the crosslinked nanogel particles potentially can affect solvent/monomer uptake (Table 3) and dispersion in direct relation to its proportional reduction of the overall matrix volume fraction [14]. As macromers, the nanogels provide network structure as well as mechanical reinforcement of secondary polymeric networks [24,28,30].

When the solvent-dispersed nanogels were mechanically tested, NG1 and NG2 notably showed significantly higher modulus after storage in water compared to their corresponding dry (solvent-free) modulus values (Table 2). For hydrogen bonding nanogel structures that do not swell excessively in water, as may be expected with internal crosslinks associated with UDMA or BisGMA, the introduction of water may provide bridging hydrogen bonding reinforcement between the urethane or hydroxyl groups, respectively, that can account for the improved wet strength observed. However, despite the higher bulk nanogel T_g found for NG1, the polymers from both NG2 and NG3 displayed significantly higher dry modulus results, probably due to the dry-state hydrogen bonding reinforcement associated with the HEMA component in these nanogels. Although all dry samples were subjected to overnight solvent evaporation in a vacuum oven, residual DMF may have also contributed to the low modulus values for NG1. DMF is a solvent with lower evaporation rate compared to ethanol. Any residual DMF present at the point of polymerization would also interfere with formation of a dense polymeric network structure.

As nano-scale gels with varying degrees of hydrophilic functional groups attached to the polymeric backbone, these materials may act like a hydrogel at a certain level. Hydrogels are polymeric materials that exhibit the ability to swell and retain a significant fraction of water within their network structure, but will not dissolve in water [31]. Nanogels cannot be dissolved, but depending on their particular solubility parameter, they can be dispersed in water or other solvents as well as monomers, with swelling that is balanced by affinity and crosslink-imposed elastic limitations. While all the polymerizations of the controls and nanogel-containing formulations reached high conversion (Table 3), it can be assumed that there would be trapped radicals that during the seven days of storage could produce some additional polymerization within these polymers. The flow of water into the polymer structure may be capable of plasticizing the polymer, weakening the strength of the interactions between chains. Residual monomers can be leached or can react into the polymer network if free radicals persist [21]. The nanogel particles are both physically and covalently retained in the final polymers with little chance for leaching.

On the other hand, the polymer produced from the solvent-based polymerization of NG3 presented a dramatic decrease in modulus when stored in water, most likely due to its high hydrophilicity as demonstrated by the relatively low water contact angle and high values of water sorption and solubility (Table 3). HEMA is a hydrophilic, water miscible monomer widely used in dental materials. It leads to rapid water uptake before and after polymerization in a wet or humid environment with deteriorating properties [32]. Furthermore, TE-EGDMA is a relatively hydrophilic cross-linker that also has ester-based crosslinks that may be hydrolytically susceptible groups when present in a highly swollen state [14]. The hydrophilic character of this NG3 polymer material could certainly contribute

to an excessive absorption of water resulting in short-term plasticization and potentially leading to longer-term hydrolytic damage.

For the polymers created in the HEMA dispersion medium, the HEMA control and all nanogels had lower flexural modulus after water storage. The poly(HEMA) control presented the lowest modulus and degree of conversion in this case. The polymerization of HEMA produces a very loosely crosslinked network that has a high affinity for water while offering little elastic resistance to aqueous swelling [32]. The substantial water uptake into the polymer overcomes the hydrogen bonding reinforcement of the many pendant hydroxyl groups and accounts for the greater loss in wet modulus compared with the other materials in this study.

Although the polymer from HEMA/NG1 also experienced a significant decrease in the value of flexural modulus when in water, the deterioration occurred in lesser magnitude. NG1 is the most hydrophobic nanogel tested, and its UDMA cross-linker absorbs less water compared to BisGMA or TE-EGDMA [14]. Furthermore, the NG1 nanogel had the highest T_g, which can be related to reduced plasticization by water and/or solute [33]. In this case, NG1 yielded reduced the water sorption and solubility of the polymeric adhesive (Table 3). UDMA is a very reactive dimethacrylate monomer that generates strong polymeric networks [26], and IBMA provides a very high glass transition temperature and hydrophobic character to the copolymer [26]. Because of its higher T_g, NG1 might have been expected to allow for lower conversion because of the lower chain mobility; however, no reduction in conversion was seen. In fact, all of the nanogel-modified resins showed enhanced conversion during polymerization. In particular, the slightly lower nanogel content used for the HEMA/NG1 formulation to normalize the viscosity may have facilitated mobility and reaction in this material. In the wet storage condition, this HEMA/NG1 sample showed significantly better mechanical properties than the other HEMA-containing compositions. It should be noted that while the dry modulus results for all the HEMA-dispersed nanogel materials were significantly greater than the dry modulus of the solvent-dispersed nanogel polymers, the wet modulus of the polymers from the solvent-dispersed NG1 and NG2 materials, which were produced with 50 wt% inert solvent, are higher than that of the wet HEMA-dispersed analogs.

For the material groups with nanogel dispersed in BisGMA/HEMA 40:60 wt%, as with HEMA, the same superior wet strength performance was observed with NG1 as the reactive additive. Despite the deterioration in stiffness after water storage, the NG1-modified resin continued to show the highest flexural modulus values. All the BisGMA/HEMA resin formulations had higher dry and wet modulus results compared with all other groups, which is probably due to the additional BisGMA cross-links in the dispersion material [14], making it both more hydrophobic and more resistant to swelling. The addition of BisGMA, a monomer of high viscosity and molecular weight, may also have hindered the polymerization due to the decreased mobility between the chains, which slightly reduced the degree of conversion, but did not compromise the material properties.

Possibly, the higher degree of conversion for the resins with NG2 and NG3 may be associated with the lower T_g's and greater mobility of chains. Nevertheless, the greater

hydrophilicity of these nanogel additives may have contributed to prejudice the values of wet flexural modulus whereas the dry modulus results are all fairly similar but lower than that of the unmodified resin control.

Thus, the null hypothesis that nanogels with different hydrophilicity would not affect the mechanical performance when exposed to water must be rejected.

While the viscosity of the nanogel-modified resins at the loading levels used here was significantly greater than that of the BisGMA/HEMA control resin, when used as solvated formulations, the handling and coating behavior of the control and experimental adhesives were similar when applied to dentin surfaces. In the 24 h microtensile bond strength analysis, ethanol-solvated BisGMA/HEMA control group and this adhesive resin with NG2 presented the highest results. It should be noted that NG2 has the same monomer composition of the adhesive resin control. BisGMA and HEMA are two of the most common monomers used in dental adhesives [14,32]. Furthermore, HEMA is long known for good wetting, diffusion, and penetration properties, which are the primary mechanisms to obtaining micro-mechanical retention on demineralized wet dentin [32]. In this way the amphiphilic character obtained with control and NG2 formulations appears to have positively influenced the interaction with the substrate. However, the predominance of mixed failure in the NG2 group compared to the predominance of adhesive failure in the control group can be an indicative of a better adhesion of the material with inclusion of nanogel. One distinction between the BisGMA/HEMA adhesive resin and the BisGMA/HEMA nanogel is that in wet bonding conditions, there is not potential for water-induced phase separation within the covalently interconnected nanogel structure.

The adhesive resin modified with NG1 and NG3 showed the lowest values of bond strength after 24 h as compared to control. The more hydrophobic characteristic of NG1 may have affected the interaction with wet dentin. An intimate contact (i.e. wettability) between the adhesive resin and the dental hard tissues plays an important role in establishing effective bonding [34]. Furthermore, considering the unfavorable mechanical performance obtained with the NG3 and the presence of solvent and water in the restorative process, these factors may have influenced the degree of conversion, strength and adhesive properties of the adhesive with the more hydrophilic nanogel. Large concentrations of hydrophilic monomers may impair the polymerization of the adhesive system and reduce the degree of conversion [10–12].

After 3 months of storage in distilled water, the group NG2 presented the best dentin bond strength results. The good performance of BisGMA/HEMA combined with NG2 appears to have contributed to a better interaction with the substrate. The enhancement in μ TBS after water storage is not completely understood but this surprising result was independently reproducible. There is a possibility that the denser polymer network structure associated with the BisGMA/HEMA resin plus nanogel may lead to more trapped radicals than in the case of the resin alone. This could result in an extended post-cure as water infiltrates the polymer structure. This outcome would not necessarily be limited to the NG2 structure but taken together with appropriate surface wetting behavior may be necessary to achieve an improving bond strength. There may also be differences in the long-term swelling character

of this nanogel-modified resin matrix that leads to more favorable interactions with the substrate. These details will certainly be explored in greater detail in subsequent studies.

The adhesive resin control and the formulations modified with NG1 and NG3 had bond strength values that were maintained after storage. It is likely that this limited 3 month time was not sufficient to obtain significant degradation of the bond strength [35]. However, the predominance of adhesive failures in all groups may indicate the first signs of degradation of this model adhesive.

Thus, the second null hypothesis that nanogels with different hydrophilicity would not interfere with dentin bond strength must be also rejected.

Dental formulations with reactive nanogels able to interact better with the substrate and to improve the hydrophilic polymerization thereby requiring no modification of conventional bonding techniques, can combine ease of use and the positive effects of stronger (mechanically reinforced) materials. This study has demonstrated that nanogel additives likely need to be tailored to specific adhesive resins and bonding conditions to achieve the most favorable results in terms of bond strength and longevity of the bonded interface. The synthesis of new nanogels will be aimed at the development of adhesive materials that allow simple dentin-bonding procedures and optimal polymerization while providing long-term stability of well-bonded interfaces. Additional studies should emphasize other aspects, such as other compositions of nanogels, the analysis of the degree of conversion at the bond interface with dentin to monitor the polymerization process over time and a longer storage time of experimental adhesives with nanogel additives.

5. Conclusion

Nanogels with different hydrophilicity were able to affect the mechanical performance of materials when exposed to water and the dentin bond strength. In general, the more hydrophobic IBMA/UDMA nanogel showed higher bulk material mechanical property results, but the best dentin bond strength values, and notably strength values that improved upon storage, were obtained with the amphiphilic nanogel based on BisGMA/HEMA.

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Fig. 1.
Failure mode analysis of the debonded specimens (%) tested by microtensile in 24 h and 3 months (m) of storage in water.

Table 1
Gel permeation chromatography parameters^a and glass transition temperature (T_g).

Nanogel sample	M _n (kg/mol)	M _w (kg/mol)	PDI	R _h (nm)	MH- α	T _g
NG1	15,460	42,760	2.76	4.76	0.360	96 °C
NG2	12,550	51,490	4.10	4.44	0.281	55 °C
NG3	3580	18,870	5.28	3.12	0.299	40 °C

^aNumber and weight-averaged molecular weight (M_n and M_w, respectively), polydispersity index (PDI), hydrodynamic radius (R_h), and Mark–Houwink α exponent (MH- α). The data represent a single analysis.

Table 2

Mean (standard deviation) of viscosity, degree of conversion and flexural modulus.

Dispersion medium	Group	Viscosity (mPa s)	Degree of conversion (%)	Flexural modulus (MPa)	
				Dry	Wet
Solvent	NG1	637.5	83.1 (3.1)b	127.6 (23.4)cB	936.7 (49.4)aA
	NG2	496.0	88.9 (3.9)a	662.5 (59.9)aB	937.1 (128.2)aA
	NG3	426.7	84.8 (5.6)b	362.7 (62.5)bA	36.7 (19.3)bB
HEMA	Control	6.0	86.3 (0.4)c	2338.0 (346.6)bA	0.3 (0.1)dB
	NG1	5984.0	90.9 (1.5)a	2485.1 (142.7)bA	512.9 (20.6)aB
	NG2	4043.7	89.3 (0.9)b	2809.6 (230.8)aA	93.6 (7.8)bB
BisGMA/HEMA	NG3	3467.2	90.9 (1.1)a	2228.1 (126.4)bA	17.9 (10.4)cB
	Control	291.8	82.9 (1.1)d	3088.8 (123.4)aA	1062.6 (46.6)bB
	NG1	6822.0	84.1 (0.8)c	2632.7 (94.2)bA	1465.3 (116.1)aB
	NG2	5757.5	91.4 (0.9)a	2546.1 (90.7)bcA	656.0 (47.7)cB
	NG3	4902.7	85.9 (1.0)b	2399.9 (131.6)cA	481.3 (40.9)dB

Mean values with the same uppercase letters (row) and lowercase letters (column) are not significantly different ($p > 0.05$; Tukey's post-hoc test).

Mean (standard deviation) of water contact angle, water sorption/solubility and microtensile dentin bond strength for control and experimental BisGMA/HEMA adhesives resins.

Table 3

Group	Contact angle (°)	Sorption (µg/mm ³)	Solubility (µg/mm ³)	Microtensile bond strength (MPa)	
				24 h	3 months
Control	73.6 (7.1)b	161.9 (5.3)a	88.3 (3.4)ab	22.5 (3.1)aA	20.4 (1.6)bA
NG1	82.6 (4.1)a	122.2 (8.6)c	78.5 (2.0)c	15.4 (0.6)bA	16.3 (4.7)bA
NG2	69.7 (3.1)bc	142.0 (1.1)b	85.0 (0.6)b	18.0 (2.1)abB	27.5 (4.9)aA
NG3	62.9 (4.3)c	166.5 (4.2)a	93.1 (4.2)a	15.4 (3)bA	16.4 (3.6)bA

Mean values with the same uppercase letters (row) and lowercase letters (column) are not significantly different ($p > 0.05$; Tukey's post-hoc test).